abcam

Product datasheet

Human Histone H3.3 (Mutated G34W, G34V, G34R) Antibody Panel ab274410

リコンビナント

画像数 21

製品の概要

製品名

Human Histone H3.3 (Mutated G34W, G34V, G34R) Antibody Panel

種交差性

交差種: Human

製品の概要

Human Histone H3.3 (Mutated G34W, G34V, G34R) Antibody Panel ab274410 contains multiple trial-sized versions of anti-human antibody clones against Histone H3.3 (Mutated G34W), Histone H3.3 (Mutated G34V) and Histone H3.3 (Mutated G34R), specifically selected for high performance in various applications. They are provided as a sampler panel to allow you to easily evaluate each antibody.

For guidelines on how to use each antibody within the panel, please consult the individual datasheet for each antibody.

Panel contains:

- Rabbit monoclonal [EPR23581-39] to H3G34W (20 µL) **ab272691**

- Rabbit monoclonal [EPR23520-5] to H3G34V (20 µL) **ab254401**

- Rabbit monoclonal [EPR23519-91] to H3G34R (20 µL) **ab254402**

特記事項

Explore our range of antibody sample panels designed to provide you with a variety of trial-size antibodies in a convenient and cost-effective format.

Directly conjugated versions of our antibodies are available and ready to use for multicolor flow cytometry or immunocytochemistry analysis. Please refer to the ‘Associated products’ section below.

Carrier-free formulations of our recombinant antibodies are also available for easy conjugation to labels of your choice and for multiplex applications. Please refer to the ‘Associated products’ section below.

製品の特性

保存方法 Store at -20°C. Please refer to protocols.

内容	1 kit
ab254402 - Anti-Histone H3.3 (mutated G34 R) antibody [EPR23519-91] -ChIP Grade	2 x 10µl
ab254401 - Anti-Histone H3.3 (mutated G34 V) antibody [EPR23520-5] - ChIP Grade	2 x 10µl
ab272691 - Anti-Histone H3.3 (mutated G34 W) antibody [EPR23581-39] - ChIP Grade	2 x 10µl

機能 Variant histone H3 which replaces conventional H3 in a wide range of nucleosomes in active genes. Constitutes the predominant form of histone H3 in non-dividing cells and is incorporated into chromatin independently of DNA synthesis. Deposited at sites of nucleosomal displacement throughout transcribed genes, suggesting that it represents an epigenetic imprint of transcriptionally active chromatin. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling.

配列類似性 Belongs to the histone H3 family.

発生段階 Expressed throughout the cell cycle independently of DNA synthesis.

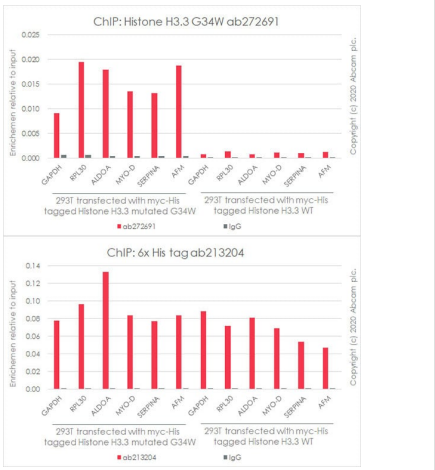
翻訳後修飾 Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me).
Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription.
Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation.
Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression.
Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at the 3' of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters.
Specifically enriched in modifications associated with active chromatin such as methylation at Lys-5 (H3K4me), Lys-37 and Lys-80. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me), which are linked to gene repression, are underrepresented. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at 'Lys-120'. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin.
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation and result in the activation of genes, such as c-fos and c-jun.
Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at

Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation at Ser-11 (H3S10ph) is also an essential regulatory mechanism for neoplastic cell transformation. Phosphorylated at Ser-29 (H3S28ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) by PKN1 is a specific tag for epigenetic transcriptional activation that promotes demethylation of Lys-10 (H3K9me) by KDM4C/JMJD2C.
Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin. Phosphorylation on Ser-32 (H3S31ph) is specific to regions bordering centromeres in metaphase chromosomes.
Ubiquitinated. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination.

細胞内局在

Nucleus. Chromosome.

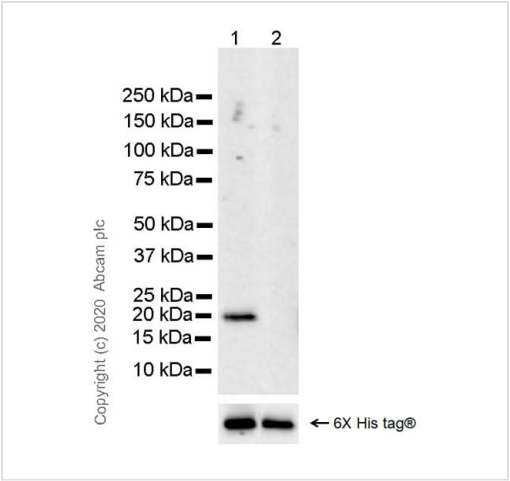
画像



ChIP - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

Chromatin was prepared from HEK-293T transfected with myc-His tagged Histone H3.3 mutated G34W and Histone H3.3 WT cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 2 µg of **ab272691** (red), or 2 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.

***https://www.abcam.com/resources?keywords=X%20ChIP%20protocol**



Western blot - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

All lanes: Anti-Histone H3.3 (mutated G34 W) antibody [EPR23581-39] - ChIP Grade (**ab272691**) at 1/1000 dilution.

Lane 1: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34W expression vector containing a myc-His-tag®, whole cell lysate, 40 ug.

Lane 2: HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 40 ug.

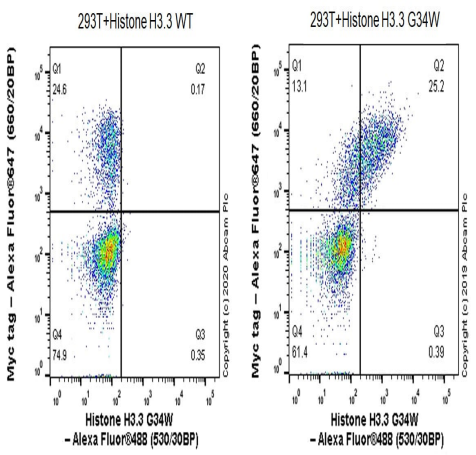
Secondary (all lanes): Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**), 1/50000 dilution.

Predicted MW: 15 kDa.

Observed MW: 20 kDa.

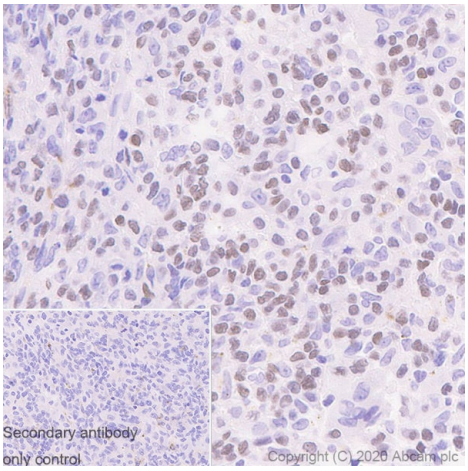
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

Flow cytometric analysis of 4% paraformaldehyde-fixed 90% methanol-permeabilized HEK-293T (Human embryonic kidney epithelial cell) transfected with myc tagged Histone H3.3 WT construct (Left panel) and myc-tagged Histone H3.3 G34W construct (Right panel) cells labelling Histone H3.3(mutated G34 W) with **ab272691** at 1/50 dilution (1µg). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

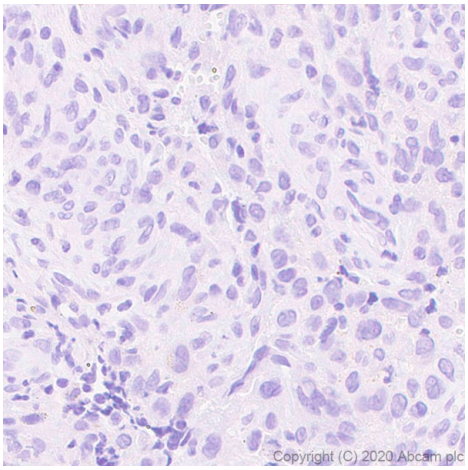


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3(mutated G34 W) with **ab272691** at 1/250 dilution followed by ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in human giant cell tumor of bone (PMID: 29757500). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

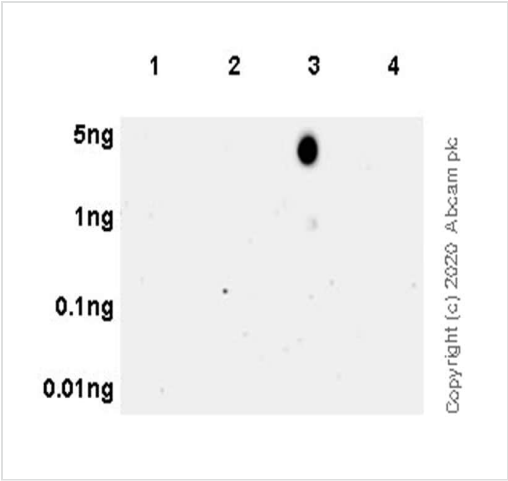
Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3.3(mutated G34 W) with **ab272691** at 1/250 dilution followed by ready to use Goat Anti-Rabbit IgG H&L (HRP).

Negative control: No staining in human chondroblastoma (PMID: 29757500).

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Dot Blot - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

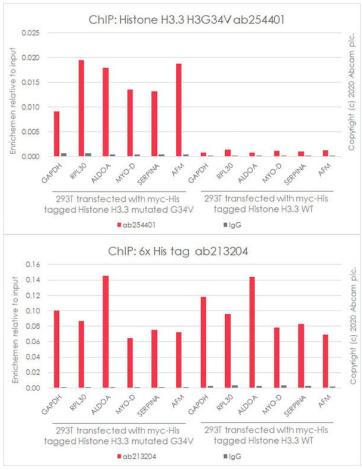
Dot blot analysis of Histone H3.3 (mutated G34 W) labeled with **ab272691** at 1/1000 dilution.

Lane 1: Histone H3.3 H3G34W peptide (aa28-37).
Lane 2: Histone H3.3 H3G34W peptide (aa33-43).
Lane 3: Histone H3.3 H3G34W peptide (aa28-43).
Lane 4: Histone H3.3 WT peptide (aa28-43).

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

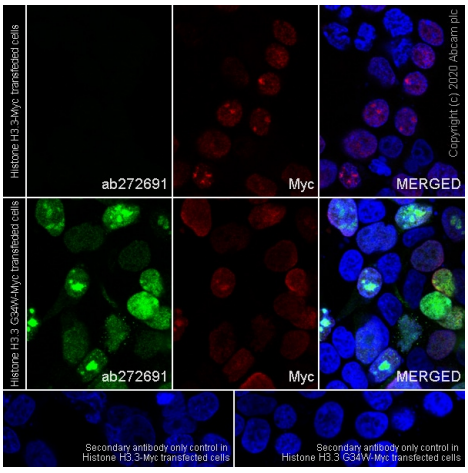
Exposure time: 3 minutes.



ChIP - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

Chromatin was prepared from HEK-293T transfected with myc-His tagged Histone H3.3 mutated G34V and Histone H3.3 WT cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with then formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 2 µg of **ab254401** (red), or 2 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.

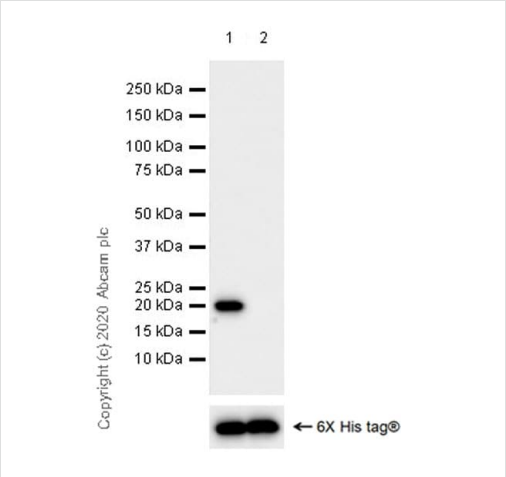
***https://www.abcam.com/resources?keywords=X%20ChIP%20protocol**



Immunocytochemistry/Immunofluorescence - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T cells labelling Histone H3.3(mutated G34 W) with **ab272691** at 1/100 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 µg/ml dilution (Green). Confocal image showing nuclear staining in HEK-293T cells transfected with Histone H3.3 G34W-Myc plasmid, while no staining in HEK-293T cells transfected with H3.3 WT -Myc plasmid. Myc-Tag Mouse mAb (Alexa Fluor® 647) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 2 µg/ml dilution.



Western blot - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

All lanes: Anti-Histone H3.3 (mutated G34 V) antibody [EPR23520-5] - ChIP Grade (**ab254401**) at 1/1000 dilution.

Lane 1: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34V expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

Lane 2: HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

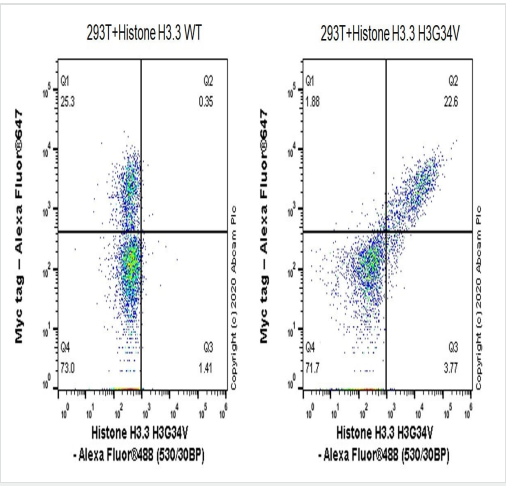
Secondary (all lanes): Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**), 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 20 kDa.

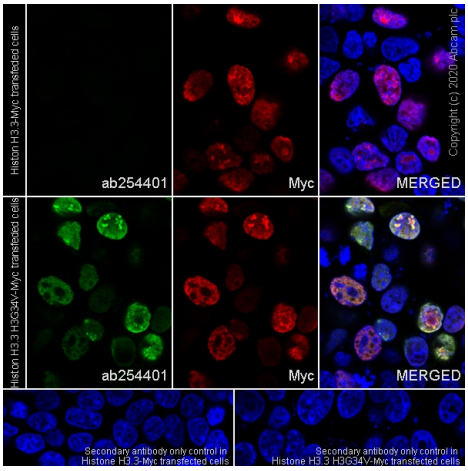
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

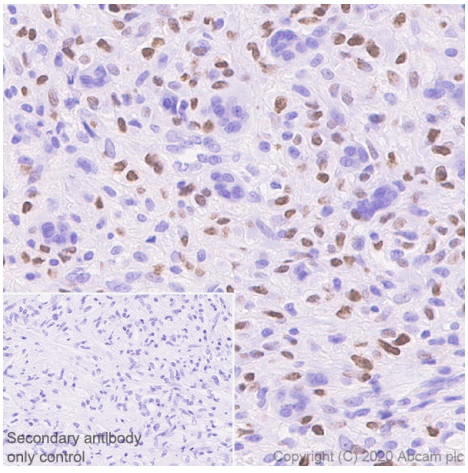
Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (Human embryonic kidney epithelial cell) transfected with myc tagged Histone H3.3 WT construct (Left panel) and myc-tagged Histone H3.3 H3G34V construct (Right panel) cells labelling Histone H3.3(mutated G34 V) with **ab254401** at 1/500 dilution (0.1µg). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/Immunofluorescence - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T cells labelling Histone H3.3(mutated G34 V) with **ab254401** at 1/1000 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 µg/ml dilution (Green). Confocal image showing nuclear staining in HEK-293T cells transfected with Histone H3.3 H3G34V-Myc plasmid, while no staining in HEK-293T cells transfected with H3.3 WT -Myc plasmid. Myc-Tag Mouse mAb (Alexa Fluor® 647) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 2 µg/ml dilution.

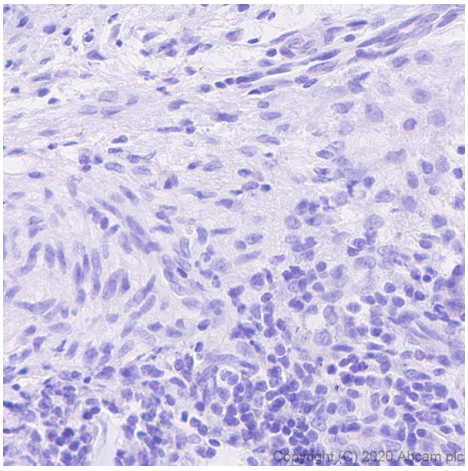


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3(mutated G34 V) with **ab254401** at 1/1000 dilution (0.542 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human giant cell tumor of bone. (PMID: 29241742). The section was incubated with **ab254401** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

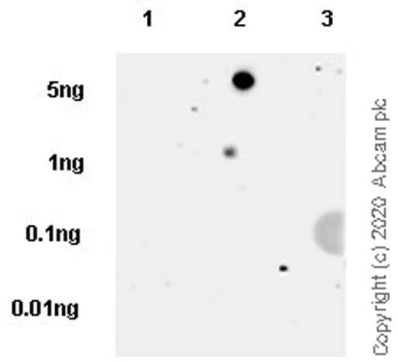
Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3.3(mutated G34 V) with **ab254401** at 1/1000 dilution (0.542 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Negative control: No staining on human chondroblastoma (PMID: 29241742).

The section was incubated with **ab254401** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Dot Blot - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

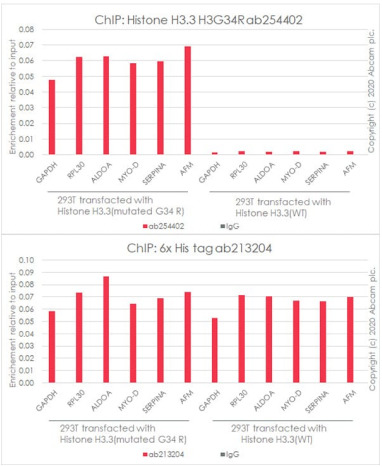
Dot blot analysis of Histone H3.3 (mutated G34 V) labeled with **ab254401** at 1/1000 dilution.

Lane 1: Histone H3.3 H3G34V peptide (aa28-40).
Lane 2: Histone H3.3 H3G34V peptide (aa26-38).
Lane 3: Histone H3.3 WT peptide (aa26-40).

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

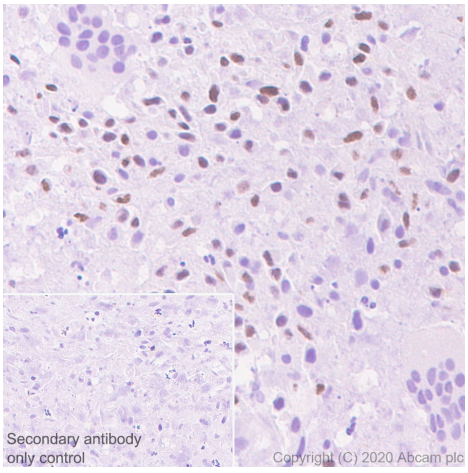


ChIP - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Chromatin was prepared from HEK-293T transfacted with Histone H3.3(mutated G34 R) and 293T transfacted with Histone H3.3(WT) cells according to the Abcam X-ChIP protocol*. Cells were fixed with formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 2 µg of **ab254402** (red), or 2 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci).

*https://www.abcam.com/resources?keywords=X%20ChIP%20protocol

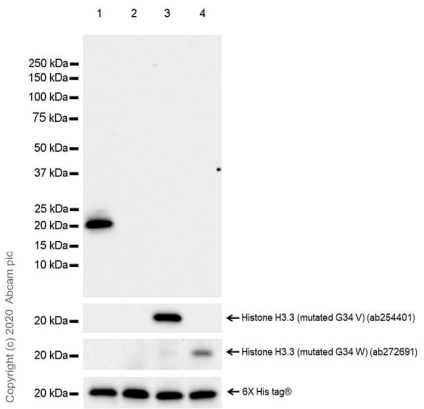


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3 (mutated G34 R) with **ab254402** at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human giant cell tumor of bone (PMID: 29241742). The section was incubated with **ab254402** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Western blot - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

All lanes: Anti-Histone H3.3 (mutated G34 R) antibody [EPR23519-91] (**ab254402**) at 1/1000 dilution.

Lane 1: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34R expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

Lane 2: HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

Lane 3: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34V expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

Lane 4: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34W expression vector containngi a myc-His-tag®,

whole cell lysate, 20 ug.

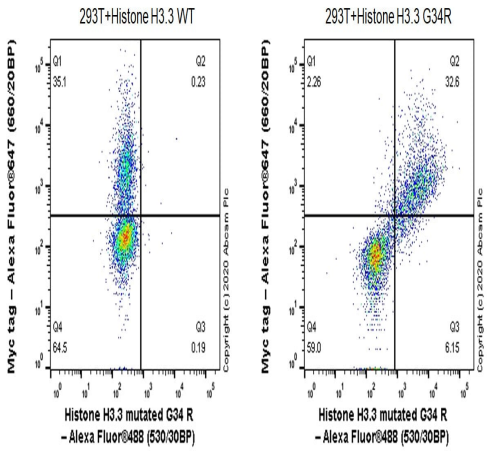
Secondary (all lanes): Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**), 1/100000 dilution.

Predicted MW: 15 kDa

Observed MW: 20 kDa

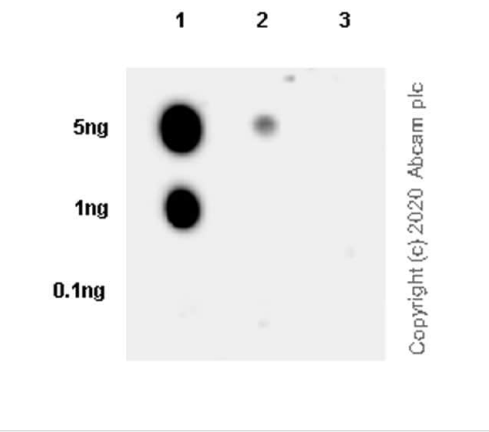
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 26 seconds.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HEK-293T (Human embryonic kidney epithelial cell) (transfected with myc-tagged Histone H3.3 WT expression vector) (Left) or myc-tagged Histone H3.3 G34R expression vector (Right) cells labelling Histone H3.3 (mutated G34 R) with **ab254402** at 1/5000 dilution (0.01ug) (Both panels). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Dot Blot - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - ChIP Grade

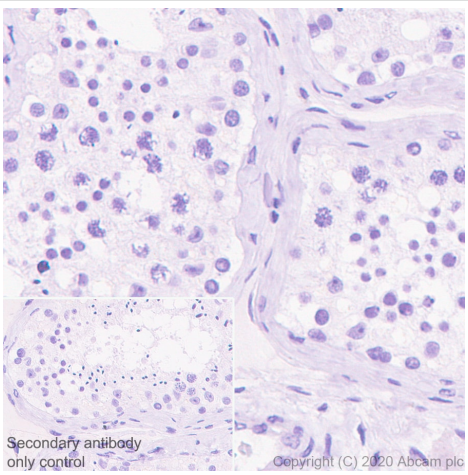
Dot blot analysis of Histone H3.3 (mutated G34 R) labeled with **ab254402** at 1/1000 dilution.

Lane 1: Histone H3.3 H3G34R peptide (aa28-40).
Lane 2: Histone H3.3 H3G34R peptide (aa26-36).
Lane 3: Histone H3.3 WT peptide (aa26-40).

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

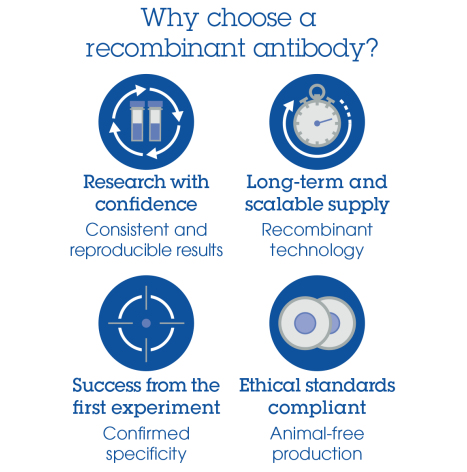


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling Histone H3.3 (mutated G34 R) with **ab254402** at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). **Negative control:** no staining on human testis. The section was incubated with **ab254402** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Human Histone H3.3 (Mutated G34W, G34V, G34R) Antibody Panel (ab274410)

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